An acyl-coenzyme A carboxylase encoding gene associated with jadomycin biosynthesis in *Streptomyces venezuelae* ISP5230

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Analysis of a region of chromosomal DNA lying between jadR and jadI in the gene cluster for jadomycin biosynthesis in *Streptomyces venezuelae* ISP5230 detected an ORF encoding 584 amino acids similar in sequence to the biotin carboxylase (BC) and biotin carboxyl carrier protein (BCCP) components of acyl-coenzyme A carboxylases. Multiple sequence alignments of the deduced Jad protein with acyl-coenzyme A carboxylases from various sources located the BC and BCCP components in the N- and C-terminal regions, respectively, of the deduced polypeptides. The organization and amino acid sequence of the deduced polypeptide most closely resembled those in other Gram-positive bacteria broadly classified as actinomycetes. Disrupting the gene, designated jadJ, severely reduced but did not eliminate jadomycin production. The disruption had no effect on growth or morphology of the organism, implying that the product of jadJ is not essential for fatty acid biosynthesis. It is concluded that jadJ supplies malonyl-coenzyme A for biosynthesis of the polyketide intermediate that is eventually processed to form the antibiotic jadomycin B.

**Keywords:** acetyl-coenzyme A carboxylase genes, jadomycin biosynthesis, *Streptomyces venezuelae*

INTRODUCTION

During growth under conditions of nutritional and environmental stress, *Streptomyces venezuelae* ISP5230 produces jadomycins, a distinctive group of polyketide antibiotics of the angucycline class (Rohr & Thiericke, 1992; Doull et al., 1993, 1994). Consistent with the fused-ring aromatic structure of jadomycin B (Ayer et al., 1991), sequence analysis of cloned polyketide synthase (PKS) genes in the cluster responsible for jadomycin biosynthesis has shown that these antibiotics are assembled by an iterative type-II PKS complex (Han et al., 1994). Biosynthetic investigations with blocked mutants (Yang et al., 1996; J. McVey, K. Kulowski, K. Yang, L. Han, C. R. Hutchinson & L. C. Vining, unpublished), and studies of polyketide chain folding and cyclization using biochemically constructed PKS gene assemblies in a compliant host (Meurer et al., 1998; Kulowski et al., 1999) indicate that the structure is derived from a C20 polyketide chain generated by condensation of an acetyl starter unit with nine C2 extender units supplied from malonyl-coenzyme A. Therefore, jadomycin biosynthesis should be associated with a strong metabolic demand for malonyl-coenzyme A.

The latter metabolite is required for chain elongation in all organisms that biosynthesize fatty acids, and its formation by carboxylation of acetyl-coenzyme A is the first committed step in the process (Alberts & Vagelos, 1972). The carboxyl group is introduced in two distinct reactions catalysed by a composite enzyme, acetyl-coenzyme A carboxylase (EC 6.4.1.2), which consists of a biotin carboxylase (BC), a biotin carboxyl carrier...
protein (BCCP) with biotin as an essential cofactor, and a transcarboxylase (TC). The initial reaction, catalysed by BC, uses bicarbonate to carboxylate BCCP; the second reaction, catalysed by TC, transfers the carboxyl group from BCCP to acetyl-coenzyme A, forming malonyl-coenzyme A (Samols et al., 1988). The organization of genes encoding the component enzyme activities of acetyl-coenzyme A carboxylase varies in different organisms; in some bacteria and chloroplasts, the activities are encoded on separate genes (Rock & Cronan, 1996; Marini et al., 1995), whereas other bacteria contain genes encoding more than one activity (Jäger et al., 1996; Donadio et al., 1996). In eukaryotes, the enzyme activities are present on a multifunctional protein transcribed from a single gene (Toh et al., 1993).

We have reported elsewhere (Kulowski et al., 1999) that two ORFs are present in the 2-4 kb EcoRI–BamHI segment of *S. venezuelae* chromosomal DNA lying upstream of and partially overlapping *jadA* in the *jadABCDE* gene cluster encoding the PKS associated with jadomycin biosynthesis (Fig. 1; Han et al., 1994; Yang et al., 1995). The ORF nearest to *jadA* is designated *jadJ*, and encodes a cyclase needed to form the angucycline ring system of jadomycins (Kulowski et al., 1999). Here we present evidence that the second ORF (designated *jadD*) encodes the BC and BCCP components of an acyl-coenzyme A carboxylase functionally associated with biosynthesis of the polyketide-derived antibiotic.

**METHODS**

**Bacterial strains and plasmids.** *Streptomyces venezuelae* ISP5230 is a wild-type strain (Stuttard, 1982) that produces jadomycins (Doull et al., 1993). *Escherichia coli* DH5α ( Gibco-BRL) was used routinely as a host for constructing recombinant plasmids based on the vector pBluescript II SK+ (Stratagene). A pBluescript II SK+ containing thiostrepton- and ampicillin-resistance genes, and partition function of its SCP2* parent, it is segregationally unstable in streptomycetes (Larson & Hershberger, 1986), and was modified by removing the KpnI site in the multiple cloning region, and is then designated pBluescript II SK+. Plasmid pHJL400, a bifunctional *E. coli–Streptomyces* vector that contains thiostrepton- and ampicillin-resistance genes, was used to disrupt genes in *S. venezuelae*; because it lacks the partition function of its SCP2* parent, it is segregationally unstable in streptomycetes (Larson & Hershberger, 1986), and is lost from 75–95% of spores of *S. venezuelae* colonies grown without selection for thiostrepton resistance (Paradkar et al., 1993).

**Culture conditions.** Spores of *S. venezuelae* ISP5230 and its transformants were harvested from cultures grown on MYM agar (Stuttard, 1982). For jadomycin production, glucose/solecine medium (Doull et al., 1994) was used. To prepare protoplasts of *S. venezuelae*, cultures were grown in MYEME medium (Hopwood et al., 1985) with the modifications described elsewhere (Yang et al., 1995). Mycelium was regenerated from protoplasts on mannitol/maltose agar (Yang et al., 1995); transformants were selected by supplementing the medium with 50 µg thiostrepton ml⁻¹ (reduced to 10 µg ml⁻¹ for shaken cultures). Recombinant *E. coli* DH5α strains were grown in LB medium containing 100 µg ampicillin ml⁻¹ (Sambrook *et al*., 1989).

**Cloning and sequencing of *jadJ*.** The cluster of genes responsible for the biosynthesis of jadomycin was obtained initially by cloning fragments of genomic DNA from *S. venezuelae* ISP5230 in the vector Lambda GEM-11 (Promega). DNA isolated from appropriate lambda clones was subcloned in the phagemid vector pBluescript II SK+ (Stratagene). A 5.0 kb XhoI fragment from lambda clone LH7 (Han et al., 1994), subcloned in phagemid pJV68A, and a 4.3 kb SacI fragment from lambda clone 8 (Ramalingam, 1989), subcloned in phagemid pJV72, encompassed the 2.4 kb EcoRI–BamHI region containing *jadJ* and *jadI* (see Fig. 1). This region was sequenced by the dideoxynucleotide procedure (GenBank accession no. AF126429), and a preliminary analysis of sequence relationships was begun. The results for *jadJ* have been reported (Kulowski et al., 1999).

**Sequence analysis.** The sequence of *jadJ* was compared with sequences in the GenBank database by using the BLASTx search program (Altschul *et al*., 1997). The deduced amino acid sequences of ACC genes were retrieved from the database, and aligned with that of *jadJ* using CLUSTAL W (Thompson *et al*., 1994). The relatedness of the aligned sequences was assessed with the PILEUP program (Devereux *et al*., 1984) of the Genetics Computer Group (GCC).

**Disruption of *jadJ*.** The segment of pJV72 DNA lying between sites for EcoRI and SacI, and containing the XhoI site, was subcloned in pBluescript II as pJV86 (see Fig. 1). An EcoRI–XhoI digest of pJV86 was then ligated with the 1.0 kb XhoI–EcoRI fragment of *S. venezuelae* DNA retrieved by agarose gel electrophoresis from a XhoI–EcoRI digest of pJV68A. pBluescript II linearized at its EcoRI site was added to the ligation mixture, which was then used to transform *E. coli* DH5α. Extraction of plasmid DNA from a suitable transformant yielded pJV87 (see Fig. 1). Plasmid pJV87 was linearized by digestion with XhoI; the ends were blunted with the Klenow fragment of DNA polymerase I in the presence of deoxyribonucleotide triphosphates, and ligated to an *E. coli* apramycin-resistance gene excised from pKC462a (Stanzak *et al*., 1986) by digestion with PstI and EcoRI as a 1.5 kb DNA fragment, and blunt-ended as above. Transformation of *E. coli* DH5α with the ligation mixture yielded pJV88 (see Fig. 1), which was sequenced using the oligonucleotide as a primer. After passage through *E. coli* ET12567, pJV88 transformed *S. venezuelae* ISP5230 to give colonies (VS665) resistant to apramycin (AmR, 50 µg ml⁻¹) and thiostrepton (TsR, 10 µg ml⁻¹). Single colonies of *S. venezuelae* VS665 were patched on MYM agar and grown without thiostrepton selection through two rounds of sporulation. Plating spores and screening by replica plating yielded colonies (VS666) with an AmR TsR phenotype.

To construct a disruption vector in which the AmR gene for insertion into the cloned *S. venezuelae* jadJ DNA was in the opposite orientation from the AmR gene in pJV88, PCR was used to amplify separately the DNA regions flanking the KpnI site in pJV87. During amplification, the 0.755 kb DNA fragment containing the 5' end of *jadJ* was modified by using 5'-GAATTCGACGGATCCCTGAAATCGGTG3' and 5'-GTTCCGGCCAGGGATATCGTCTCCACGTTGCCGCGC3' as forward and reverse primers, respectively. In this way, an EcoRV site was introduced after KpnI at the 3' end of the amplified DNA (EcoRV and BamHI recognition sequences in the primers are underlined), allowing the amplified fragment to be cloned by ligation to the BamHI site in pJV87. After digestion of pJV87 with EcoRV and BamHI, the amplified DNA fragment was ligated to the EcoRV–BamHI fragment of the 2.4 kb EcoRI–BamHI region containing *jadJ* and *jadI* (see Fig. 1). This region was sequenced by the dideoxynucleotide procedure (GenBank accession no. AF126429), and a preliminary analysis of sequence relationships was begun. The results for *jadJ* have been reported (Kulowski et al., 1999).
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Fig. 1. (a) Sacl restriction map of the 20 kb segment of *S. venezuelae* ISP5230 genomic DNA cloned in the recombinant Lambda GEM11 vectors lambda 8 and lambda LH7. Broad arrows above the map indicate the genes identified and their orientations. Numbers shown below the map are the sizes (in kb) of the Sacl fragments. The location of the core PKS gene cluster (Han et al., 1994) is shown. (b) Plasmids used in subcloning the jadomycin biosynthesis gene cluster from the recombinant lambda vectors LH7 and 8, and also in constructing the disruption vectors pJV88 and pJV111. Inserts cloned in pJV68A, pJV72 and pJV86 are shown; the restriction sites correspond to those in the restriction map in (a). Restriction sites: B, *BamHI*; E, *EcoRI*; K, *KpnI*; P, *PstI*; S, *SacI*; X, *XhoI*. Sites marked by an asterisk in pJV88, VS665 and VS666 were destroyed during blunt-end ligation. The arrowhead above Am R in the cross-hatched DNA segment indicates transcriptional orientation of the gene conferring apramycin resistance.

and *EcoRV* sites in the multiple cloning region of pBluescript II·SK+. The recombinant plasmid isolated after transformation of *E. coli* DH5α contained the *BamHI–KpnI* segment of pJV87, and was designated pJV87L. It was subsequently linearized with *KpnI–ClaI* to provide the vector DNA for construction of pJV110 (see later). The *KpnI–EcoRI* end of pJV87, containing 1·1 kb of DNA from the 3′-region of jadJ, was amplified by PCR using 5′-GGGCGGGCGATA-TCTTCTGGAACATGCAAGAAG-3′ and 5′-CGGGTGGATCGATAGGCGCGGCGGGCGGGGCGGTTA-AAGCTTAGTCCCTTG-3′ as primers to replace the *KpnI* and *EcoRI* sites with *EcoRI* and *HindIII*, respectively, and to introduce a *ClaI* site outside *HindIII* at the 3′ end (*EcoRI, HindIII* and *ClaI* recognition sites in the primers are underlined). The modified sequence amplified from the 1·1 kb *KpnI–EcoRI* segment of pJV87 (originating from the 3′-region of jadJ) was cloned in pBluescript II·SK+ to give pJV87R (see Fig. 1). The insert was retrieved from a *BamHI–ClaI* digest of the plasmid to obtain a DNA fragment with a *BamHI* site outside *EcoRI* at the 5′ end, and a *ClaI* site outside *HindIII* at the 3′ end.

Transformation of *E. coli* DH5α with a ligation mixture consisting of a *HindIII–KpnI* digest of pJV87L that included the pBluescript II·SK+ vector fragment obtained after digestion of pJV87L with *BamHI* and *ClaI* (see above), a *KpnI–BamHI* digest of pJV85 (containing the apramycin-resistance gene recloned from pKC462a in pBluescript II·SK+), and a *BamHI–ClaI* digest of pJV87R, yielded colonies from which plasmid pJV110 was extracted. Digestion of pJV110 with *HindIII*, and agarose gel electrophoresis of the products, furnished a 3·35 kb fragment that was cloned in the *HindIII* site of pHJL400 to give the disruption vector pJV111, carrying the *AmR* gene in the *KpnI* site of pJV87.

Hybridization. DNA from agarose electrophoresis gels was transferred to nylon membranes by standard procedures (Hopwood et al., 1985), and probed for hybridization to DNA fragments labelled with digoxigenin-dUTP (DIG) by the
random priming procedure. The membrane was washed with 0.1× SSC (0.015 M NaCl in 1.5 mM sodium citrate, pH 7.0) containing 0.1% SDS at 65 °C. The washing stringency was estimated to allow hybridization only between nucleotide sequences with 70% or greater similarity. Signals were detected by a chemiluminescence procedure (Boehringer Mannheim).

**Jadomycin production and analysis.** Strains of *S. venezuelae* were initially grown for 24 h in 25 ml Trypticase soy broth (3%, w/v; BBL)/glucose (1%, w/v) medium. A 1 ml portion of the culture was used to inoculate each 25 ml of galactose/isoleucine production medium (Doulil et al., 1994) contained in a 250 ml Erlenmeyer flask. For the single-crossover transposition of *jadA* (1%), *S. venezuelae* were each supplemented with 1 µg thiostrepton ml⁻¹. At 6 h the production cultures were each supplemented with 10 ml ethanol; at 1, 3 and 5 d, filtered samples (5 ml) of the production cultures were extracted with equal volumes of ethyl acetate, and the extracts were evaporated. The residue from each extract was redissoled in 200 µl methanol and analysed by HPLC with Beckman programmable solvent and detector modules 126 and 166, respectively, controlled by System Gold software. Samples (20 µl) were injected (model 210 injector) into a Phenomenex Columbus C18 column (50×4.6 mm, with a 10 mm guard column) and chromatographed with linear gradients of solvent A (0.1% trifluoroacetic acid in 50% methanol and acetonitrile) and solvent B (0.1% trifluoroacetic acid in acetonitrile). The solvent programme (flow rate 1.0 ml min⁻¹) was: 0–3 min, 100% A to 70% A:30% B; 3–6.5 min, 70% A:30% B to 100% B; 6.5–8 min, 100% B; 8–10 min, 100% B to 100% A; 10–12 min, 100% A, which re-equilibrated the column for the next injection.

**RESULTS**

**Nucleotide sequence of *jadJ***

The sequence of a 2.4 kb EcoRI–BamHI segment of *S. venezuelae* DNA overlapping by 21 bp the 5′ end of *jadA* was detected from a region of the chromosome cloned in the lambda vector GEM-11. The *jad* gene cluster was detected by hybridization with the act PKS genes of *Streptomyces coelicolor* A3(2) (Malpartida et al., 1987; Han et al., 1994), and fragments were identified from a restriction map of the region subcloned for sequencing in the phagemid pBluescript II. **CODON PREFERENCE** analysis (Devereux et al., 1984) of the 2.4 kb EcoRI–BamHI segment located two ORFs, assigned to *jadl* and *jadJ*. Both ORFs were oriented in the same direction as the PKS genes, with that for *jadl* lying next to *jadA* (Kulowski et al., 1999); *jadJ* was 112 nt upstream of *jadl* and remote from *jadA* (see Fig. 1). A GTG at nt 153–155, allocated as the putative start codon for *jadJ*, was preceded (11 nt spacing) by a plausible ribosome-binding site (GGAG). The first in-frame stop codon was a TGA at nt 1905–1907. Thus *jadJ* is predicted to encode 584 aa. The polypeptide is calculated to have an *M*₉ of 61926 and an isoelectric point of 4.91.

**Comparison with acyl-coenzyme A carboxylase genes***

A search of the GenBank protein database using BLASTX showed high degrees of similarity between the deduced aa sequence of *jadJ* and sequences recorded for acyl-coenzyme A carboxylases (ACCs). The closest overall

resemblances were to the BC/BCCP components of propionyl-coenzyme A carboxylase in the erythromycin producer *Saccharopolyspora erythraea* (Donadio et al., 1996; 70% identity), to the BC/BCCP components in acetyl-coenzyme A carboxylase from *Mycobacterium leprae*, and to these components in a probably bifunctional acetyl/propionyl-coenzyme A carboxylase from *Mycobacterium tuberculosis* (Norman et al., 1994; both 68% identity). There was also marked similarity (61% identity) to the sequence deduced for presumptive BC/BCCP components of a gene cloned from *Corynebacterium glutamicum* (Jäger et al., 1996). Each of these gene products from Gram-positive bacteria in the actinomycete family aligned closely with the JadJ sequence (Fig. 2), but in other respects as well, this group of gene products has much in common; one such feature is their organization into a bifunctional protein with BC and BCCP domains located in the N-terminal and C-terminal regions, respectively. The amino acid sequences deduced for the domains are quite similar to the corresponding ACC subunits from other organisms. The two-thirds of JadJ nearest the N terminus resembles the BC component of other enzyme complexes. Consistent with other BCC proteins, the JadJ sequence has a glycine-rich region extending from positions 164 to 167, and a conserved cysteine at position 231 (see Fig. 2). The sequence in the C-terminal one-third of the *jadJ* product exhibited similarities to sequences in the BCCP component of acetyl-coenzyme A carboxylases. These included the pentapeptide motif EAMKM, which is the biotin-attachment site conserved in these BCCPs, and recognized in the *jadJ* product at aa 549–553 (see Fig. 2).

A pileup alignment of the deduced amino acid sequence of the BC domain encoded by *jadJ* with the BC subunits of acetyl-coenzyme A carboxylase from various organisms, and analysis of relationships, showed that on the basis of sequence similarity, BCs from the actinomycete family of Gram-positive bacteria group together, and have diverged from the BCs of other bacteria. The BCs in *Archea* (e.g. *Sulfolobus metallicus*) or *Eubacteria* (e.g. *E. coli*) are encoded by a single discrete gene. In this respect as well as in sequence similarity, the BC sequences from *Chlamydia trachomatis*, several plants and a *Synechococcus* sp. group with archaeal and eubacterial species. Presumably in the plants this reflects the plastid source of the acetyl-coenzyme A carboxylase gene sequence. The eukaryotic (chicken, rat and yeast) BC sequences examined could be distinguished from those of bacteria in that each constituted one domain in a multifunctional protein exhibiting all of the activities of acetyl-coenzyme A carboxylase (Toh et al., 1993). Analysis of sequence relationships also placed the BCs of eukaryotes in a distinct group well separated from the BCs of bacteria.

Alignment and analysis by pileup of sequences from the BCCP components of *S. venezuelae* JadJ and of acetyl-coenzyme A carboxylases from other organisms for which data were available from GenBank accessions showed relationships parallel to those found for the BC components.
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Fig. 2. Alignment by CLUSTAL W (Thompson et al., 1994) of the amino acid sequences deduced from jadJ of S. venezuelae (JadJsv.) and the genes for biotin carboxylases from: Corynebacterium glutamicum (Coryjglu.), Mycobacterium leprae (Mjlepr.), Mycobacterium tuberculosis (Mj tuber.) and Saccharopolyspora erythraea (Sacjery.). Highly conserved amino acids are indicated by asterisks. The cysteine residue important for biotin carboxylation is indicated by an arrow. The pentapeptide is boxed and the lysine residue serving as a biotinylation site is underlined.

Disruption of jadJ

Assembling in the EcoRI site of pHJL400 adjacent EcoRI–XhoI and XhoI–EcoRI fragments that had been subcloned separately from the primary lambda vectors generated a vector (pJV87; see Fig. 1) in which the jadJ sequence was present as a 1–85 kb EcoRI–EcoRI insert of S. venezuelae DNA. This construction allowed the recombinant plasmid to be readily linearized at the centrally located XhoI site, blunt-ended and ligated to a similarly blunted E. coli gene for apramycin resistance (Stanzak et al., 1986). Transformation of E. coli DH5α with the ligation mixture yielded the disruption vector pJV88, which was resolated from the DNA-methylation-deficient E. coli strain ET12567 to avoid restriction during subsequent transformation of S. venezuelae. A single colony (VS665) resistant to both thiostrepton and apramycin was chosen; cultures of VS665 grown without thiostrepton selection in galactose isoleucine medium optimized for jadomycin 907
B production resembled wild-type *S. venezuelae* in their growth characteristics, but accumulated only 10–13% as much jadomycin B. Southern hybridization of EcoRI-digested chromosomal DNA from AmR TsR transformants using the 1·9 kb EcoRI fragment of pJV87 as a DIG-labelled probe gave a strong signal at 3·4 kb, and a weaker signal at 1·9 kb; no hybridization was detected at 9·1 kb. The results suggested that these transformants were mixtures in which pJV87 had integrated by a single crossover but in a majority of strains had subsequently undergone a second crossover; in the absence of thiostrepton selection the segregationally unstable free plasmid regenerated by the second crossover had been largely eliminated (Fig. 1). This expectation was supported by the preponderance of TsR colonies present when spores from VS665 cultures propagated without thiostrepton selection were replica-plated on agar media supplemented with thiostrepton and apramycin or apramycin alone. An AmR TsR strain (VS666) was readily isolated; by Southern hybridization of EcoRI-digested chromosomal DNA using the conditions described for VS665 it gave only one signal, at 3·4 kb, confirming that the parent jadJ had been replaced with the disrupted gene, and that no free vector was present. The VS666 mutants produced somewhat less jadomycin B than did the initially isolated AmR TsR transformants (4–7% versus 10–13% of wild-type titres).

Sequencing a segment of pJV88 DNA containing a junction between the AmR gene and jadJ verified that the AmR gene was inserted at the blunted XhoI site, and showed that it was oriented for transcription in the opposite direction to jadJ. Because the loss of jadomycin production could, therefore, be due to a polar effect on transcription downstream of jadJ rather than to selective loss of JadJ activity, a second disruption vector (pJV111) was constructed. To place the AmR gene in the same transcriptional orientation as jadJ, the jadJ sequences on each side of a central KpnI site in the vector pJV87 (together these adjacent segments constituted the cloned *S. venezuelae* DNA containing jadJ; see Fig. 1) were amplified separately by PCR using primers designed to introduce additional distinctive restriction sites. The two PCR fragments were trimmed by digestion with appropriate restriction enzymes and ligated, along with a DNA fragment carrying the AmR gene, into a modified pBluescript II vector. Restriction sites on all three fragments and the vector favoured insertion of the AmR gene at the KpnI site in jadJ, and mandated the desired orientation (see Fig. 1). Transformant colonies of *E. coli* DH5α incubated with the ligation mixture yielded pJV110, the insert from which was recloned in the HindIII site of pHJL400 to give the disruption vector pJV111. This was passaged through *E. coli* ET12567 and used to transform *S. venezuelae* ISP5230 as before. The AmR TsR colonies obtained were treated as putative single-crossover mutants; screening for vector elimination associated with a second crossover after release from thiostrepton selection yielded the expected AmR TsR colonies (VS674). When BamHI digests of VS674 genomic DNA were probed by Southern hybridization with a DIG-labelled 0·755 kb HindIII–KpnI fragment of pJV111, a single strong signal was obtained at 2·25 kb, as expected for a double-crossover mutant (see Fig. 1). Cultures of VS674 grew normally on minimal agar; in galactose/isoleucine liquid medium optimized for jadomycin B production, they resembled cultures of VS666 in showing depressed titres of the antibiotic (2–11% of the wild-type level).

**Discussion**

Although jadJ is located in the gene cluster associated with jadomycin B biosynthesis in *S. venezuelae* (Han et al., 1994; Yang et al., 1995), it has convincing similarity in organization and deduced amino acid sequence to genes encoding two subunits of the ACCs that initiate fatty acid biosynthesis in many organisms. The N-terminal amino acid sequence of the jadJ product bears a resemblance to BCSs, and the C-terminal region includes sequences highly conserved in BCCP components of ACCs. The *S. venezuelae* gene most closely resembles those described in three other high-mol% G+C Gram-positive bacteria (*Sac. erythraea*, two *Mycobacterium* species and *C. glutamicum*) in possessing a fused gene encoding a putative bifunctional BC-BCCP with separate domains for each activity. A gene cloned as part of a cluster of PKS genes from another high-mol% G+C Gram-positive species, *Saccharopolyspora birma*. was reported to code only for BCCP, and was closely flanked upstream by a gene for PKS ketoreductase rather than by a gene encoding BC or any predictable ACC activity (Le Gouill et al., 1993). Whether this BCCP gene functions selectively in the secondary metabolic PKS pathway shown by Le Gouill and colleagues to be present in *Sac. birma* has not been established.

Differences in ACC sequence parallel differences in organization of the ACC-encoding genes, and reflect differences in evolutionary history (Samols et al., 1988; Toh et al., 1993). In *E. coli*, the enzyme activities that convert acetyl-coenzyme A to malonyl-coenzyme A for fatty acid biosynthesis are present in an ACC that consists of four subunits, each protein encoded by a separate gene (one each for BC and BCCP, and two for the different TC activities; Rock & Cronan, 1996). In contrast, eukaryotic organisms contain all of their ACC activities in a single large polypeptide (Toh et al., 1993). In the actinomycete family of high-mol% G+C Gram-positive bacteria, where a fused BC/BCCP gene generates these subunit activities in one polypeptide (Norman et al., 1994), a second subunit contains TC activity. The separate evolutionary histories evident from analysis of BC sequence similarities confirm the assignment of JadJ to the fused BC/BCCP group, but indicate that it diverged from other members at the outset of the group’s diversification. Conceivably this might mark the time at which the gene cluster for jadomycin biosynthesis recruited an auxiliary enzyme dedicated to the secondary metabolic process. In another situation potentially casting light on the origin of secondary metabolic
pathway genes, B. subtilis 168 contains a gene yotE (reported as yngH in GenBank) encoding a BC that is presumed to function in the biosynthesis of fatty acids needed to form the lipopeptide antibiotic fengycin (Tosata et al., 1997). The gene yotE is present in an operon with yotD, which encodes an acyl-coenzyme A ligase; moreover, yotE shows only 52% sequence identity with the BC gene (accC) associated with fatty acid synthesis in Bacillus subtilis (Marini et al., 1995). Based on its sequence and gene organization (it encodes only BC activity), yotE belongs to the diverse group of bacterial ACCs that includes the primary metabolic accC gene of B. subtilis, and it may be significant that here too the secondary metabolic yotE shows early divergence from the group, implying again that secondary metabolic pathways have existed since ancient times.

Copies of jadJ disrupted by inserting an apramycin-resistance gene in either orientation, and reintroduced into S. venezuelae via the disruption plasmids pJv88 and pJV111, gave mixtures of single- and double-crossover mutants at frequencies indicating that disruption of the native gene was not lethal. This result contrasts with reports that reduced expression of acetyl-coenzyme A carboxylase in a BC/BCCP conditional mutant of B. subtilis blocked fatty acid biosynthesis and decreased both growth and sporulation (Perez et al., 1998). In E. coli, a temperature-sensitive mutation (fabE) in the BCCP gene became lethal above 30 °C (Li & Cronan, 1992). Possible lethality was also suggested (Jäger et al., 1996) as an explanation for the failure of all attempts to isolate a mutant disrupted in the BC/BCCP gene of Corynebacterium glutamicum by double crossovers with a disruption plasmid. The facile disruption of jadJ in S. venezuelae without a detectable effect on growth implies that this gene is not essential for fatty acid biosynthesis, and that a second ACC gene meeting the requirements of primary metabolism is present in the organism.

The severely reduced yield of jadomycin B after gene replacement by a double crossover, irrespective of the orientation of the apramycin-resistance gene, indicated that the jadJ product has a major role in biosynthesis of the polyketide intermediate that is processed to form jadomycin B. Retention of some jadomycin production when the plasmid had integrated by a single crossover could be attributed to expression from the single intact copy of jadJ still present. It is probably significant that production was lower after the second crossover, but that complete loss of jadJ activity did not diminish jadomycin biosynthesis to zero. The residual 10–15% of normal jadomycin titres produced by JadJ-disrupted mutants may be due to metabolic leakage from the primary biosynthetic enzyme, but it is apparent that the primary jadJ homologue is unable to fully complement a defective secondary metabolic jadJ. One possible reason might be the temporal difference in expression of primary and secondary metabolic pathway genes (Chater & Bibb, 1997), but a more direct role in channelling malonyl-coenzyme A into polyketide biosynthesis is likely.

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